

TWENTY-FIVE Plant Calcification

HOWARD J. ARNOTT*

I. INTRODUCTION	609
II. THE CALCIUM CYCLE	610
III. TYPE AND LOCATION OF MINERALS	612
IV. CYTOLOGY AND ULTRASTRUCTURE	617
V. SCANNING ELECTRON MICROSCOPY	624
VI. NONOXALATE CALCIFICATION	624
References	626

I. INTRODUCTION

Deposits of calcium salts are abundant (even massive) in or on many plants. Perhaps the most common calcium deposits are the raphide crystals of calcium oxalate found in the cells of many higher plants. Leeuwenhoek described the needle shaped raphides of *Arum* in 1675, and it has long been a common schoolboy's (or Professor's) prank to ask an uninitiated person to taste the corm of the Jack-in-the-pulpit (a species of *Arum*). The first bite is usually the last, since the raphides cause an intense burning sensation. Pohl (1) reports that severe contact dermatitis may occur from the raphides of *Ornithogalum*, and I have experienced a similar reaction to the mucilaginous exudate from the century plant (*Agave*) which contains many raphides. Such distaste, in fact, forms the basis for one of the functions often attributed to calcium oxalate deposits, especially raphides, namely, that the crystals

* Department of Biology, University of South Florida, Tampa, Florida.

“discourage” insects, snails, and other animals from eating the plants. Despite the common distribution of calcium deposits in almost all groups of plants, and even though they have many positive relationships to man and other animals, little is known about the exact mechanism(s) by which these or other calcium deposits are formed in plants, and no general agreement has been reached as to the function(s) of calcified tissues or their relationship to metabolism or physiology.

For reviews of literature on plant calcification the reader may refer to the following: Haberlandt (2), Netolitsky (3), Pobeguín (4), Küster (5), Arnott and Pautard (6).

The term calcification can be used with reference to plants in the same sense as in animals (6), namely, an accumulation of the mineral in tissues, no distinction as to the form of the mineral need be made. The inorganic deposits may be crystalline, noncrystalline aggregates, or merely a diffuse impregnation with calcium.

II. THE CALCIUM CYCLE

Metabolically calcium is essential to the growth of all plants. While some fungi (7) and a few algae (8) can survive on small concentrations of calcium, most plants must take in substantial amounts of the mineral for normal growth. In the algae the optimum level of calcium may be keyed to the nature of the cell wall present (9) and in land plants calcium deficiencies are often associated with defects in the cell wall (10). In addition to its importance in the wall, calcium is an important constituent of membranes, microtubules, and other cellular components; the ability of calcium to covalently link substances of diverse natures is of critical functional importance.

The formation of calcium deposits is, of course, dependent on the amount of calcium present in the soil or growth medium. In this regard the tolerance of plants to calcium as well as the minimal amounts required for growth are important considerations. The removal of calcium from a complete hydroponic solution has a drastic and rapid effect on the seedlings of the sunflower and other plants. Lack of calcium causes a disintegration of the terminal growing portions of the shoot in many species, the first symptoms of which may be the malformation of the youngest leaves. It is a common experience of both horticulturists and field botanists that some species will thrive on calcareous soils (calcicoles) whereas the other plants are absent or their growth is retarded (calcifuges) by such soil. It is clear that the requirements for calcium can vary widely, even between two closely related species (11), but it is also important to note that the amount of calcium has

CALCIUM CYCLE IN PLANTS

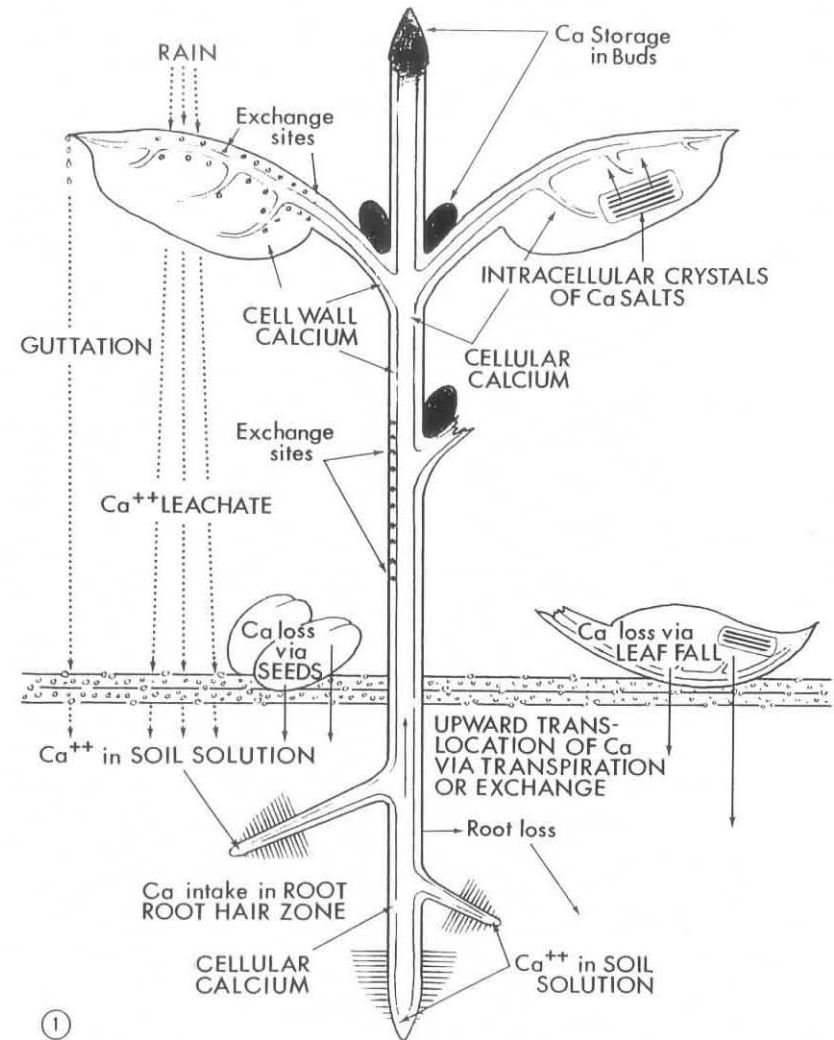


FIGURE 1. The calcium cycle in plants. A generalized scheme which indicates the major interrelationships of calcium in higher plants. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

considerable influence on the absorption of other ions, especially magnesium.

The absorption, translocation, and loss of calcium in higher plants follows well-defined routes. In Fig. 1 we see that calcium in the soil is taken up primarily in the roots (12), although under experimental conditions it can also be taken up from foliar spray (13). In general, the movement of calcium through the plant is believed to be through the transpiration stream, although the movement may be complicated and slowed, in comparison to the transpiration stream, by exchange processes (14). The remobilization of calcium has been reported in some cases, for example from old leaves to young leaves (15), in the reabsorption of druses during flowering (16), by the disappearance of crystals in ripening seeds (17), in the leaf of *Vanilla* as it develops (18), but not in other cases (19).

Calcium is lost from plant tissues through decay, abscission, guttation, and leaching. Guttation, the exudation of water containing dissolved substances from leaf surfaces, is a normal and widespread phenomenon in higher plants (20). Both organic and inorganic substances are lost through guttation, and calcium is one of the major constituents lost via this process. Rain or mist will leach calcium as well as other minerals from leaves; Mecklenberg and Tuckey (21) found that up to 40% of the calcium absorbed (as ^{45}Ca) was lost in 4 days of continual leaching by mist spray. The loss of calcium by decay of organs still attached to the plant represent an important avenue of calcium loss, especially in the root system. The loss of leaves, flowers, and other organs by abscission represents another avenue of loss which is especially important in deciduous plants; while some minerals are withdrawn from the organs to be detached, this is not true for calcium, boron, manganese, and silicon which are usually left in the tissues to be abscized.

Absorption, translocation, mobilization, and loss constitute a calcium cycle in plants. Many aspects of this cycle remain to be investigated. Some of the features of the cycle are illustrated in Fig. 1.

III. TYPE AND LOCATION OF MINERALS

Crystals of oxalate represent the most common calcium salt in seed plants; these deposits are generally intracellular and often occur in cells called idioblasts (Figs. 2-5). The crystals may be single and massive (styloids); single (or twin) prisms or pyramids (prismatic); needle shaped in packets of as many as 2000 crystals per cell (raphides), multiple "star-shaped" (druse), or in fine crystals (crystal sand). Less commonly intracellular deposits of calcium carbonate are also found, usually they are amorphous (Fig. 6) but they may sometimes be crystalline (22). Carbonates are more commonly

TABLE 1. DISTRIBUTION OF CRYSTALLINE INORGANIC SALTS IN PLANTS^a

Calcium Salt	Angiosperms	Gymnosperms	Pteridophyta Bryophyta and Relatives	Algae	Fungi
Oxalate	Found in most species. Not found in Cyperaceae and some members of Scrophulariaceae. Usually typical for each species	Found in most species	Found in fern leaves. Rarely found in Bryophyta	Reported occasionally	In hyphae of some subjects. Relatively little present
Carbonate	Found commonly in cell walls, heartwood, and occasionally in leaves. Aragonite in fruit of <i>Celtis</i> . Also as amorphous deposits in many families	Reported in cell walls	Found in cells of some species	Widely distributed	In hyphae, plasmodia, and fruiting bodies of many species
Sulfate	In pith of some plants		Reported in one or two subjects	Present in the Cyanophyceae	
Silicate	In association with silica (possibly) in cystoliths		In Equisetales?		
Citrate Tartrate Malate	Commonly found in fruits (e.g., pericarp), in seed epidermis, and in some leaves				Products of fermentation of some yeasts
Phosphate	Reported in leaves, stems, and heartwood of a few species				

^a Slightly modified from H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

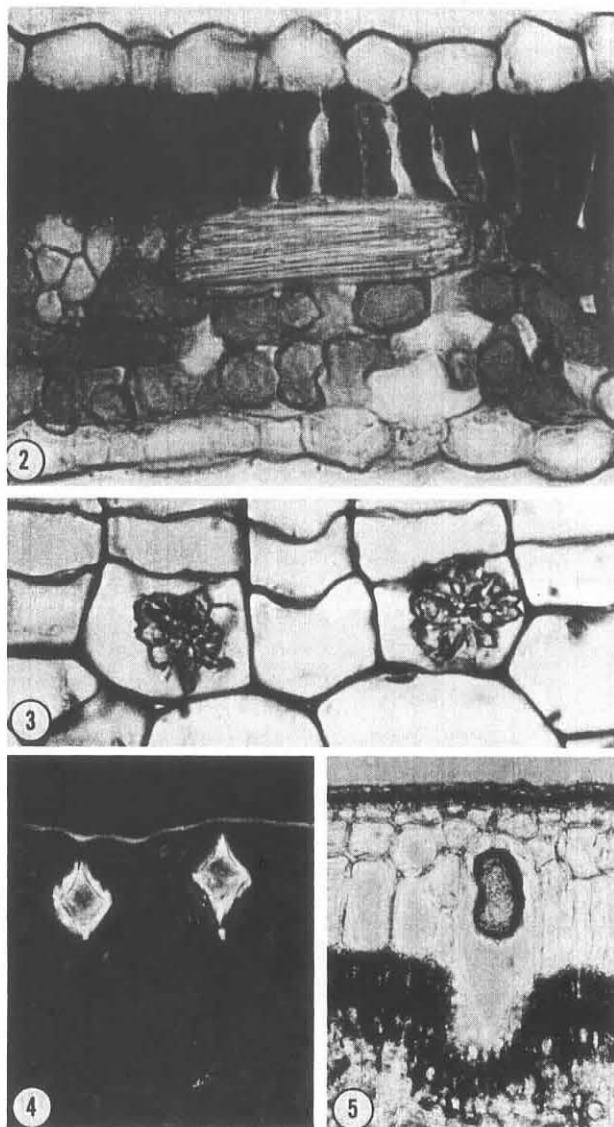


FIGURE 2. A raphide idioblast in the leaf of the grapevine showing many crystals of calcium oxalate. Note the difference in size between the idioblast and other cells. $\times 473$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

associated with the surface of various uni- or multicellular algae, especially red and green marine forms; they are also associated with certain groups of fungi, for example, the slime molds. Calcium sulfate, phosphate, silicate, citrate, tartrate, and malate salts are found in rare cases, for example, the calcium sulfate crystals in the terminal vacuoles of the desmids. The distribution of crystalline inorganic salts in plants is given in Table 1.

The crystallographic nature of many of these compounds, especially the oxalate, has been investigated by x-ray diffraction both through powder patterns (4, 6, 23) and by single crystal analysis (23); Smith *et al.* (22) have recently reported on the carbonate in hackberry (*Celtis*) fruits; through powder pattern analysis they found that 99+ % of the intracellular crystalline material was composed of aragonite. The aragonite contained 810 ppm Sr, only one-third as much Sr as reported for algal calcite and less than one-tenth the Sr found in coralline aragonite.

In plants three broad sites of calcification can be defined: (a) metabolic areas which may be temporarily rich in calcium, (b) structural zones in association with features like the wall, and (c) crystalline inorganic salts or amorphous (Fig. 5) deposits that are retained over long periods in characteristic, and sometimes idioblastic, cells. We will consider only the last category; see Arnott and Pautard (6) for a discussion of the former topics.

Crystalline deposits may be located in the cell wall of higher plants (23, 24); however, they are most often found as intracellular deposits. The deposits are one of the most characteristic cytological features of plant cells and a substantial amount of botanical literature is devoted to them (2, 5, 6, 25).

The distribution of calcium oxalate containing crystal cells within plants is of considerable interest, even though the physiological significance of these patterns is not yet understood. For example, in the leaves of *Ginkgo biloba* two size classes of crystals are associated with the veins; large crystals found on every side of the vein in the vein sheath parenchyma and small ones found only in the phloem parenchyma (6). In this case their position indicates that the crystal containing cells may have some interrelationship with the transpiration system, but whether this is direct or secondary is not clear.

FIGURE 3. Druses (Multiple cluster of calcium oxalate crystals) in the stem of the geranium. $\times 531$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

FIGURE 4. Prismatic crystals of calcium oxalate in the leaf of the Seville orange as seen under crossed nicols. $\times 423$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

FIGURE 5. Cystolith (amorphous deposits of calcium carbonate) in the leaf of the rubber plant. $\times 664$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

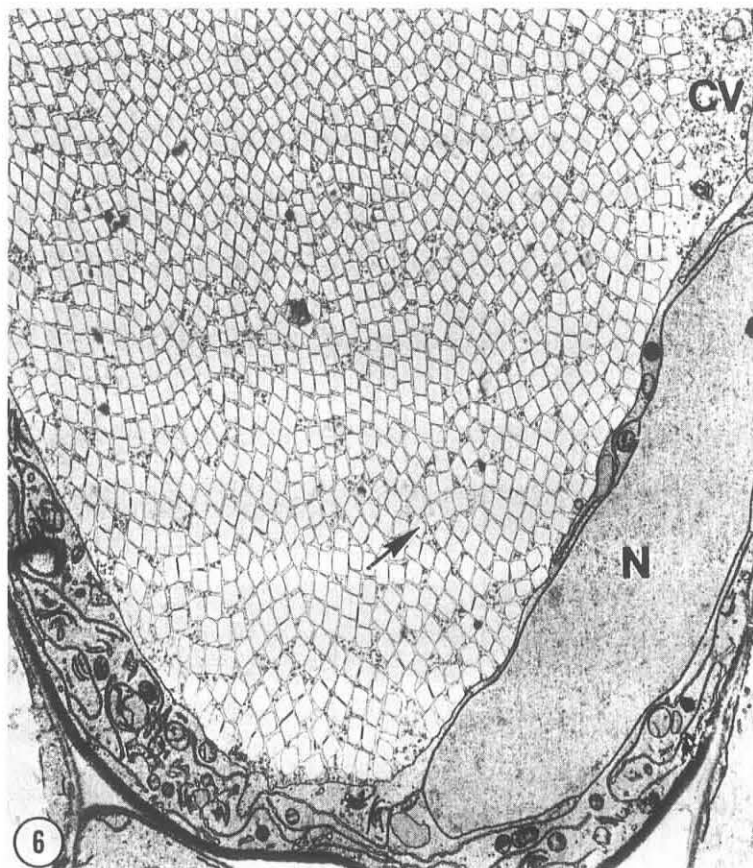


FIGURE 6. An electron micrograph showing a transverse section of a raphide idioblast in the water hyacinth. The large number of crystal chambers (arrow) in the crystal vacuole represent the site where the calcium oxalate crystals were. Processing has extracted the crystals. (N = nucleus; CV = crystal vacuole). $\times 5900$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

In *Eichhornia crassipes*, the water hyacinth, three types of crystal cells occur but their distribution in the plant is not uniform; styloids are common in the leaf and in some parts of the inflorescence but they are absent in the inflorescence nodule and in the root where only raphides are found. In *Yucca*, styloids and raphides are common in the leaf but only raphides are found in the root and in the flowers (6, 26). Large druses are found in the cortical parenchyma of *Opuntia* and other cacti (see Figs. 10–13) whereas only smaller, less-pointed druses are found in the epidermal cells of the same organ (27). Such a list could be continued but it is beyond the scope of the present paper.

IV. CYTOLOGY AND ULTRASTRUCTURE

At the optical level the cells involved in the intracellular deposition of calcium salts may have no special features that separate them from their neighbors; however, they are more often quite different, especially in size; crystal idioblasts are usually larger. The idioblasts may have dimensions 10 times that of the surrounding cells; this size disparity seems equally true for cells containing calcium oxalate crystals as for those that contain amorphous deposits of calcium carbonate (see the illustrations in Ref. 28).

The ultrastructure of calcium oxalate crystal containing cells has been examined by Arnott and Pautard (23), Esau (29), Arnott (30), Mollenhauer and Larson (31), Wattendorf (32), Thurston (33), Arnott and Pautard (6), Schotz *et al.* (34), Frank and Jensen (35), Ledbetter and Porter (36), Horner and Whitmoyer (37), Brackenridge and Arnott (38), and Price (42). Altogether about 16 plants have been reported on: 1 gymnosperm, 8 dicots, and 7 monocots, a rather small sample from approximately 300,000 described species of higher plants.

From these it is possible to make some generalizations about the oxalate crystal containing cells. In all except one case (in *Larix*, Ref. 32) the crystals are produced and contained within a vacuole(s), designated as a crystal vacuole by Arnott (30) (Fig. 6). The individual crystals are found with their own crystal chamber (30) whether the cell contains 1 or 2000; the crystal chambers appear to be formed by membranes or membrane-like layers, and it is within these chambers that the calcium oxalate crystals develop (Figs. 6–8). The chambers are interrelated with different types of membranes, tubules, or fibrils, either to one another or between one another and the cytoplasm (Fig. 7). The system may be highly organized and interconnected as in *Lemna* (6, 30) and *Spirodela* (36) or the chambers may show little or no relationship to each other. Chambers are present whether the crystal form is that of a raphide, styloid, or druse.

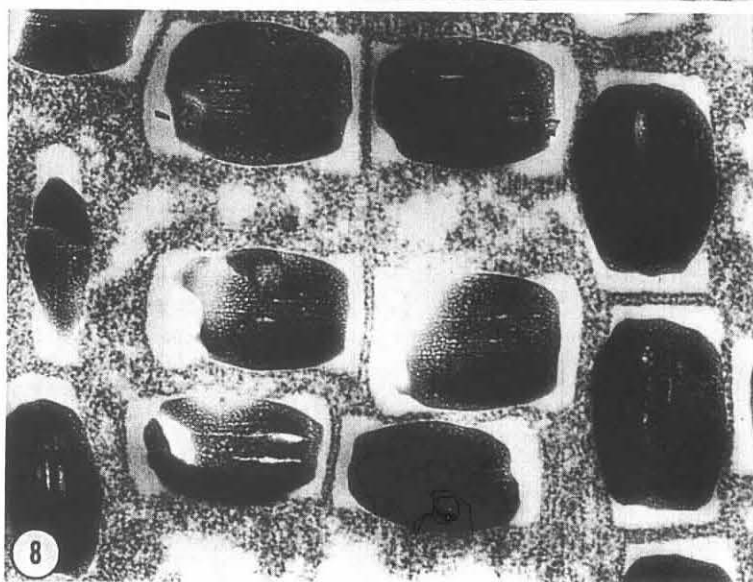
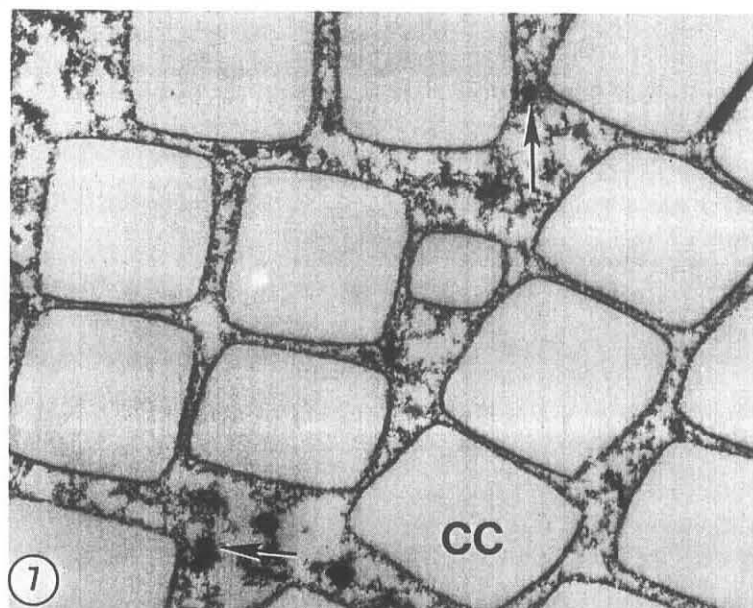


FIGURE 7. Crystal chambers (CC) of the water hyacinth. Note tubules (arrows) between the chambers. Calcium oxalate extracted from chamber during processing. $\times 66,000$.

Let us now consider the various organelles contained within the crystal containing cells. First, the wall in most cases is much like that found in the other cells in the area (Fig. 6). In certain plants, however, at a late stage of development, an outgrowth of the wall may secondarily surround the crystal. Frank and Jensen (35) describe a wall of this sort in the jack bean; in this case the wall develops in such a way that the crystal is exteriorized, that is, the crystal becomes completely surrounded by cellulosic wall materials at which time the crystal is found outside the plasma membrane. A similar phenomenon has been described in nose burn (*Trageia*, a member of the nettle family) (33). Küster (5) reviewed this condition; the wall material surrounding the crystal has been reported as cellulose (16), lignified cellulose (35), and suberin (32).

In the century plant (*Agave*) as the styloid crystal cells mature the cell wall appears to collapse around the crystals. In this case the cell wall often takes on the shape of the crystals (Fig. 9). Although some photoplasmic remnants are still visible at this time, it is not clear whether these cells are dead or what possible functional significance this collapse may have. The leaf itself is still alive and would live for many years.

A Golgi apparatus composed of many dictyosomes is generally found in crystal cells; Frank and Jensen (35) report that numerous dictyosomes, each with many vesicles, occur in the younger crystal cells of the jack bean. They have been clearly present, although not in special abundance in the several genera that I have examined. Dictyosomes are particularly noticeable in the developing raphide cells of *Oenothera* as illustrated by Schotz *et al.* (34). Here vesicles produced by the Golgi apparatus and the endoplasmic reticulum are apparently involved in the buildup of the crystal chambers.

Mitochondria and endoplasmic reticulum are both present in what would be considered normal amounts for plant cells, that is, they are present in about equal amounts in crystal and noncrystal containing cells. Frank and Jensen (35) pointed out that although similar numbers of mitochondria and dictyosomes are present in crystal and noncrystal cells, the amount of cytoplasm is much greater in the crystal cells; therefore, perhaps a quantitative difference exists between the two kinds of cells in regard to the total number of organelles present. Both Mollenhauer and Larson (31) and Schotz *et al.* (34) found a buildup in endoplasmic reticulum, the latter authors presented evidence that indicated a direct relationship between endoplasmic

FIGURE 8. Crystal chambers in the root of *Yucca* in which much of the calcium oxalate has been retained during processing. Lines in crystals may represent zones of higher water content that have exploded as the result of the electron beam. $\times 58,000$. From H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), © 1970 by Meridith Corporation. See ref. 6.

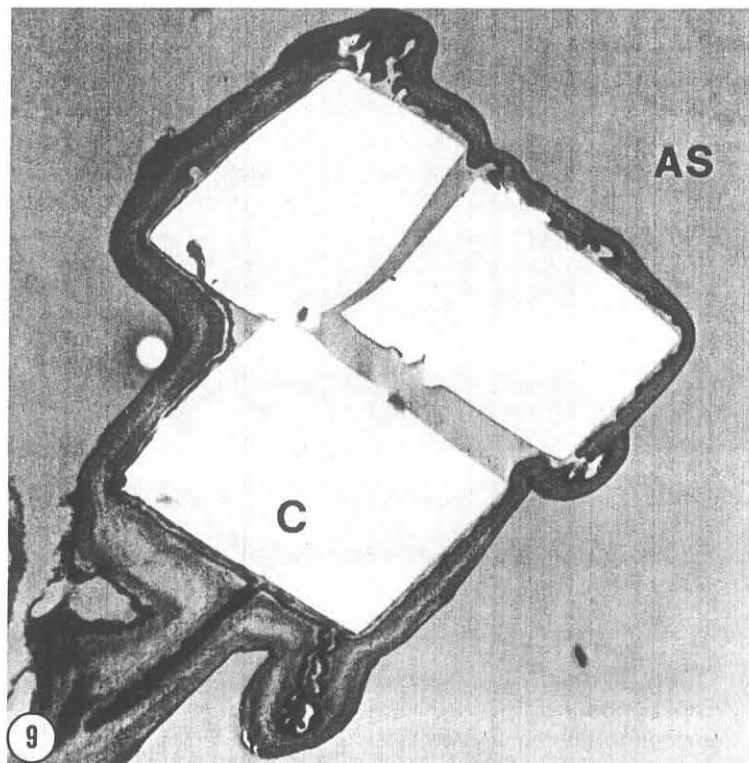


FIGURE 9. Cross section of three styloids in a crystal cell of the century plant. The cell wall has collapsed around the crystals (C), a small amount of cytoplasm is seen between the cell wall and the crystals. The crystal idioblast is within an intercellular airspace (AS) in the leaf. $\times 3000$.

reticulum vesicles and developing crystal chambers. Both smooth and rough endoplasmic reticulum or endoplasmic reticulum vesicles were common in *Spirodela* (36).

In certain plants the plastids are very different in the crystal containing cells, so much so that they were designated crystalloplastids by Arnott (30). In *Yucca* large crystalloplastids containing few membranes are only present in the raphide cells of the root. These plastids have large electron lucent areas with KMnO_4 fixation; these areas probably represent zones in which some material was stored but which has been leached from the tissue during the fixation process. In the other cells of the root, normal proplastids

similar to those seen in many other monocots are found. A distinction between crystal cell plastids and those found in other cells also has been seen in cacti. On the other hand the distinction between the crystal and noncrystal cell plastids of *Lemna* was not nearly so distinct; they may be slightly smaller and contain fewer membranes but otherwise they were alike. A similar condition was reported (35, 42); in the jack bean it was also reported that the plastids in the crystal containing cells appear to be restricted to certain areas within the respective cells.

The difference in plastid structure may be of some significance, especially in green tissues of various organs. Nagahisa and Hattori (39) were able to show an intense oxalic acid oxidase activity in the leaves of *Chenopodium*. According to their research this activity was "firmly bound" to the chloroplasts. Perhaps we could speculate that chloroplasts, or normal plastids, are necessary for the production of oxalic acid oxidase; cells without "normal" plastids might become crystal cells because of an inability to produce oxalic acid oxidase and hence the buildup of oxalic acid and eventually calcium oxalate crystals.

Frank and Jensen (35) found no evidence that microbodies are especially numerous in crystal cells; this is important in view of the possible role of microbodies in the production of oxalic acid. They did find, however, that there seemed to be a buildup of protein in the crystal cells. This is interesting because Bornkamm (40) has shown that high oxalate production is strongly correlated with high protein production.

The crystal vacuole is surrounded by a discrete tonoplast in most of the cases studies. Within the vacuole the crystal chambers are located; in the chambers the crystals are found. A crystal idioblast was found in a water hyacinth leaf which contained 2140 crystals (6, 30); in most crystal cells each crystal remains separate from all others. Apparently the individuality of the crystals is maintained by virtue of the chamber structure. Each chamber is formed by a membrane; in some cases this membrane seems to have the dimensions and structure of a unit membrane. Dense material can often be seen on the noncrystalline side of the chamber membrane.

When calcium oxalate crystals are isolated from the leaf of *Agave*, they can then be demineralized by treatment with EDTA or other agents. After careful treatment the chamber membrane remains; the membrane is PAS positive, indicating it contains a polysaccharide component, and it retains the original shape of the crystal even after demineralization; if the crystal had facets, then the chamber membrane will have facets.

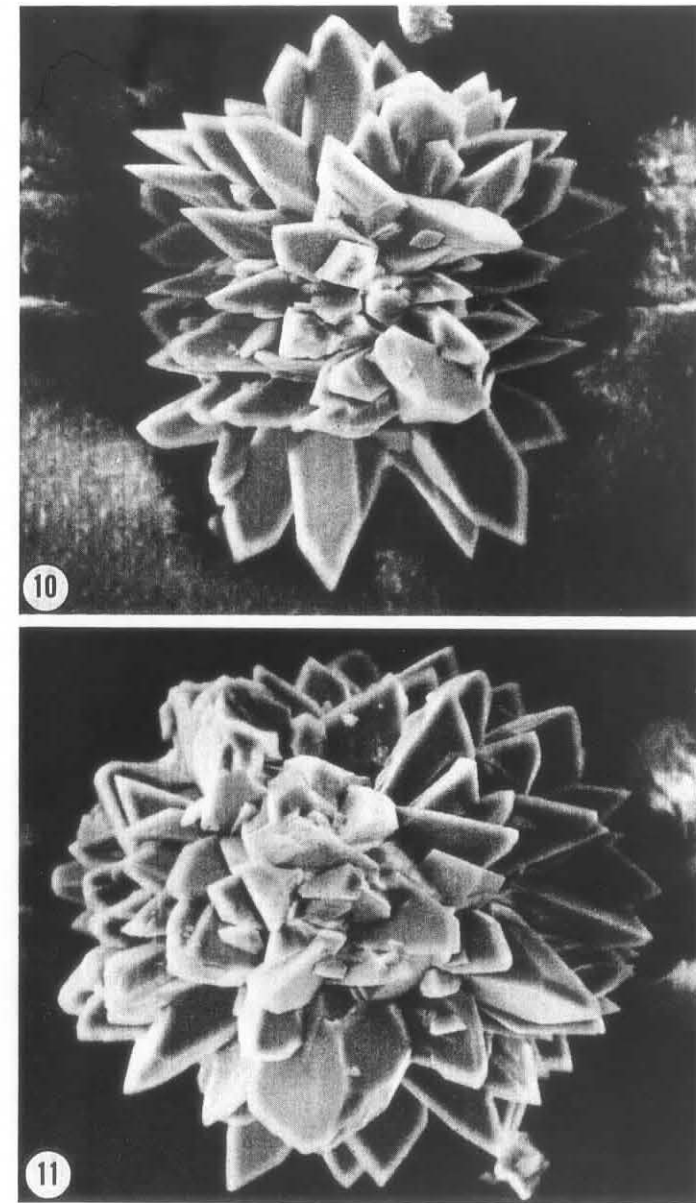
The origin of the chamber membrane is obscure; however, Schotz *et al.* (34) point to the possible transformation of cytoplasmic vesicles (derived from endoplasmic reticulum and dictyosomes) into the angular crystal cham-

bers. They also clearly state that the formation of the chambers precedes that of the crystals. Ledbetter and Porter (36) also found the chambers (which they called raphidosomes) came before the crystals. Chambers first, crystals second, seems to be the rule.

It has been suggested that the chambers contain a matrix in which calcium oxalate can crystallize (6, 30, 34). It might further be suggested that the inner surface of the chamber membrane, or the matrix contained within, represents a nucleation site where crystallization can occur. Ledbetter and Porter (36) suggest that the chamber membrane may contain a calcium pump while the membrane is permeable to oxalate. It is also possible to suggest that the tubules, membranes, fibrils, and vesicles associated with the crystal chambers inside the vacuole might be a mechanism which transports calcium from the cytoplasm to the chambers, a kind of plumbing system which delivers calcium to the chambers (already loaded with oxalate) where the crystals can form.

Whatever the mechanism is that brings calcium and oxalate together, it seems clear that the crystal chambers and vacuole play important roles in the process. In this connection it is important to point out that in an entirely unrelated system, fish use essentially the same mechanism to produce crystals within their reflecting cells (43). In the ratfish skin, intracellular crystals of guanine function in the reflection of light, a phenomenon that is part of their camouflage. The crystals are each formed within a chamber that is found within a crystal vacuole; connections between the cytoplasm and the chamber membrane are commonly found. The over-all system is identical to that found in plants. Also, further unpublished studies have shown that the chambers arise first and only after they are formed do the guanine crystals develop.

It seems clear that the process of mineralization, at least as it concerns the formation of calcium oxalate crystals in plants, is an event under close cellular control. The cell dictates where the mineral will be formed, it dictates the shape of the crystal formation, and it dictates the number of crystals that will be formed. X-Ray diffraction studies have shown that the crystalline material in the druses formed in the leaf of the castor bean (*Ricinus*) is exactly the same as that in raphides formed in the root of *Yucca* or in the styloids found in the leaf of *Agave* (6). In all three cases, calcium oxalate monohydrate is the crystalline species. One mineral substance but many forms, apparently controlled by the cells involved. While the number of crystals formed may vary, each plant (or tissue) seems to produce its crystals in a constant pattern. Plants may possess a genetic "blueprint" of each crystal before it is deposited, and its growth is thus decided by biological rather than by physical factors.



FIGURES 10 and 11. Scanning electron micrographs of druses isolated from the stem of *Opuntia* (a cactus). Numerous component crystals make up this druse. Note the sharp facets exhibited by the component crystals. Fig. 10, $\times 350$; Fig. 11, $\times 350$.

V. SCANNING ELECTRON MICROSCOPY

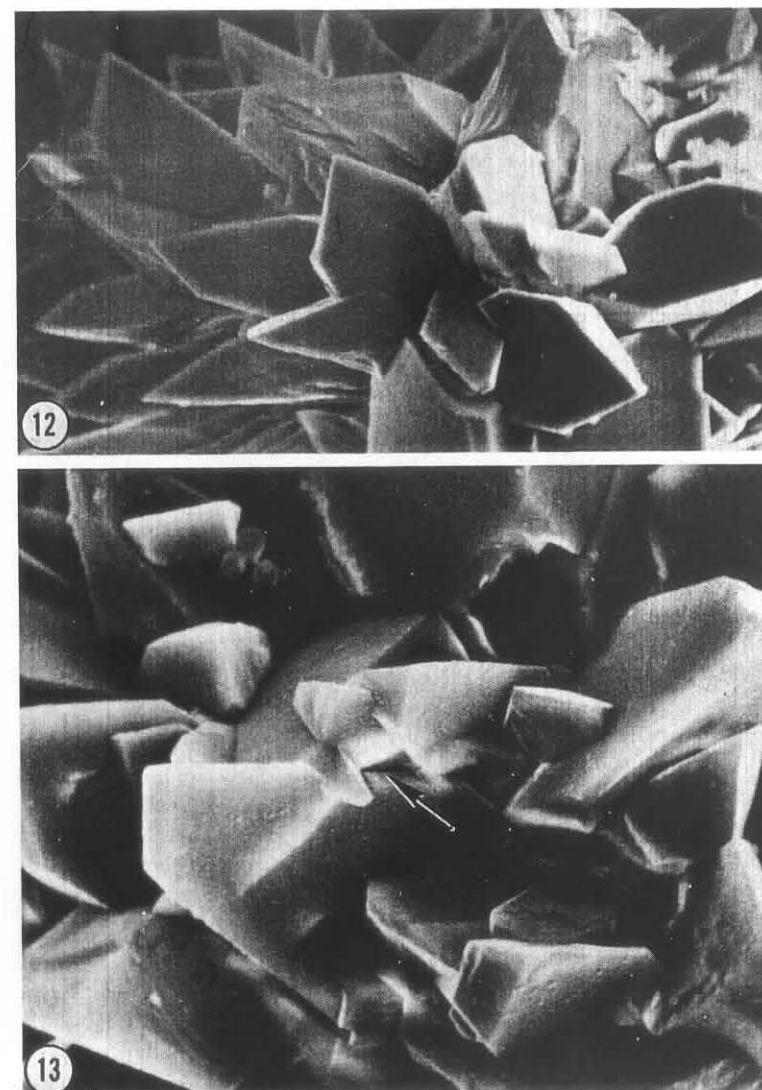
The use of the scanning electron microscope in the study of plant calcification appears to have great potential. For example, we have long been hindered by a lack of understanding of the nature of druses. Figures 10–13 illustrate what can be seen using the scanning electron microscope. In this case the druses were extracted from the stem of cactus by Mr. E. R. Rivera and studied in our laboratory. These micrographs give us a much greater understanding of the complex nature of these structures than can be gained with any other methods. Optical and transmission electron microscopy are hampered by sectioning; however, the great depth of field of the scanning electron microscope provided unique information about these objects. In these micrographs we can examine the number, shape, and distribution of the component crystals that make up the druse. In the druse in Fig. 10 there are over 80 visible crystals, while in the druse in Fig. 11 there are over 100 component crystals visible. It is clear, even from preliminary studies, that the shape, disposition, number, and size of the component crystals in druses is highly variable. Clearly, some component crystals arise on surface facets of other crystals (Figs. 10 and 11). Perhaps the most fascinating micrographs are those which clearly show the interlocking nature of some component crystals (Figs. 12 and 13).

Previous work on druses indicates that the crystals forming a druse, like those of other types, develop within crystal chambers. The inter-relationship between the various chambers, however, was very difficult to understand. Examination of the scanning electron micrographs begins to supply an understanding of these components. How the interlocking crystals develop or whether the individual crystals are separate or not has not yet been determined.

The scanning electron microscope offers a tool that should enhance our understanding of calcification in plants many fold. It will provide us with information that can help in understanding the development and function of these deposits, but perhaps more important, it will allow the development of comparative crystal morphology as a viable taxonomic, developmental, and functional tool.

VI. NONOXALATE CALCIFICATION

As can be seen in Table I, nonoxalate crystalline deposits are widespread in plants, especially in certain algal groups. A few angiosperm families are rich in amorphous calcium carbonate deposits in which the carbonate may occur diffusely in the cell or may be found in large stalked bodies called cystoliths



FIGURES 12 and 13. Scanning electron micrographs of druses isolated from the stem of *Opuntia* (a cactus). Note the interrelationships between the component crystals, especially the manner in which they interlock (arrow). Fig. 12, $\times 350$; Fig. 13, $\times 1350$.

(Fig. 5). In these intracellular bodies the carbonate appears to be deposited on a silica impregnated extension of the cell wall. Cystoliths occur in the common rubber plant (*Ficus elastica*) where they arise as epidermal idioblasts (Fig. 5).

Crystalline deposits of calcium carbonate are often found on the surface of aquatic plants in both fresh and sea water. However, perhaps the most important plants in this regard are the marine forms, the red and green algae, which often have thick deposits of calcium carbonate on their surface. An electron microscopic study of *Halimeda* (a marine green alga) showed that the aragonite crystals begin to form on the plant at about 36 hr of age and continued to increase in number as the plants aged until they became white (41). This study found that the crystals were random in their orientation on the surface of the plant and that they were not associated with any organic matrix.

In conclusion it is obvious that it is not possible to cover the field of plant calcification in a short article such as this. It can be pointed out, however, that much work is still required before we have anything approaching a unified picture of plant calcification. Important in this regard are studies on the physiological role of calcium, the possible structural (skeletal) significance of calcification, and finally the role that cells play in the formation of characteristic crystalline and amorphous deposits.

ACKNOWLEDGMENTS

I wish to thank Dr. W. Gordon Whaley for his encouragement in our studies of biological crystals and for his support in the proposition that animals and plants do have something in common. I also wish to thank Mr. R. Ramirez-Mitchell and Mr. E. R. Rivera for their technical assistance. This work was supported in part by various grants from The Graduate School of The University of Texas at Austin for which the author is most grateful.

REFERENCES

1. R. W. Pohl, *Toxicon*, **3**, 167 (1965).
2. G. Haberlandt, *Physiological Plant Anatomy*, MacMillan, London, 1914.
3. F. Netolitzky, in *Handbuch der Pflanzenanatomie* (K. Linsbauer, ed.), Gebrüder Borntraeger, Berlin, 1929.
4. T. Pobeguín, *Ann. Sci. Nat. Bot., Ser. 11*, **4**, 1 (1943).
5. E. Küster, *Die Pflanzenzelle* (K. Höffer and G. Küster-Winkelmann, eds.), Gustav Fischer, Jena, 1956.
6. H. J. Arnott and F. G. E. Pautard, in *Biological Calcification* (H. Schraer, ed.), Appleton-Century-Crofts, New York, 1970, p. 375.
7. R. A. Steinberg, *Science*, **107**, 423 (1948).
8. J. B. Walker, *Arch. Biochem.*, **60**, 264 (1956).
9. J. C. O'Kelley and W. R. Herndon, *Science*, **130**, 718 (1959).
10. E. J. Hewitt, *Plant Physiol.*, **38**, 155 (1963).
11. E. G. Bollard and G. W. Butler, *Ann. Rev. Plant Physiol.*, **17**, 77 (1966).
12. G. Laties, *Ann. Rev. Plant Physiol.*, **10**, 87 (1959).
13. M. J. Bukovac and S. J. Wittwer, *Plant Physiol.*, **32**, 428 (1957).
14. C. W. Bell and O. Biddulph, *Plant Physiol.*, **38**, 610 (1963).
15. P. A. Vlasjuk and A. M. Grodzinskii, *Trans. Referat. Zh. Biol.* **52843**, Byul. Po. Fiziol. Rast., **2**, 38 (1958).
16. F. M. Scott, *Bot. Gaz.*, **103**, 225 (1941).
17. A. Niethammer, *Planta Med.*, **12**, 53 (1931).
18. D. Brunezema, *Arch. Pharm. (Weinheim)*, **266**, 86 (1928).
19. O. Biddulph, F. S. Nakayama, and R. Cory, *Plant Physiol.*, **36**, 429 (1961).
20. S. S. Ivanoff, *Bot. Rev.*, **29**, 202 (1963).
21. R. A. Mecklenberg and H. B. Tukey, Jr., *Plant Physiol.*, **39**, 533 (1964).
22. B. Smith, G. K. Hoops, L. L. Land, and R. L. Folk, *Naturwissenschaften*, **58**, in press.
23. H. J. Arnott, H. Steinfink, and F. G. E. Pautard, *Nature*, **208**, 1197 (1965).
24. I. W. Bailey and C. G. Nast, *J. Arnold Arbor.*, **29**, 77 (1948).
25. C. R. Metcalfe and L. Chalk, *Anatomy of Dicotyledons*, Clarendon Press, Oxford, 1950.
26. H. J. Arnott, *Univ. Calif. Publ. Bot.*, **35**, 1 (1962).
27. E. Rivera, personal communication.
28. S. J. Carlquist, *Comparative Plant Anatomy*, Holt, Rinehart and Winston, New York, 1961.
29. K. Esau, *Hilgardia*, **37**, 17 (1965).
30. H. J. Arnott, in *Third European Symposium on Calcified Tissues* (H. Fleish, H. J. J. Blackwood and M. Owen, eds.), Springer-Verlag, Berlin, 1966, p. 152.
31. H. H. Mollenhauer and D. Larson, *J. Ultrastruct. Res.*, **16**, 55 (1966).
32. J. Wattendorf, *Z. Pflanzenphysiol.*, **60**, 307 (1969).
33. L. Thurston, Ph.D. Thesis, Iowa State University, Ames, 1969.
34. F. Schötz, L. Diers, and H. Bathelt, *Z. Pflanzenphysiol.*, **63**, 91 (1970).
35. E. Frank and W. A. Jensen, *Planta*, **95**, 202 (1970).
36. M. Ledbetter and K. Porter, *Introduction to the Fine Structure of Plant Cells*, Springer-Verlag, New York, 1970.
37. H. T. Horner, Jr., and F. Whitmoyer, *J. Cell Sci.*, in press.
38. J. Brackenridge and H. J. Arnott, *Tex. Rept. Bio. and Med.*, in press.
39. M. Nagahisa and A. Hattori, *Plant Cell Physiol.*, **5**, 205 (1964).
40. R. Bornkamf, *Flora (Jena)*, **156**, 139 (1965).
41. K. M. Wilber, L. H. Colinaux, and N. Watabe, *Phycologia*, **8**, 27 (1969).
42. J. L. Price, *Amer. J. Bot.*, **57**, 1004 (1970).
43. H. J. Arnott and J. A. C. Nicol, *Can. J. Zool.*, **48**, 137 (1970).